THE MECHANISM OF THE METHANE FERMENTATION

BY

FRANK WINCENT SOLLO, JR.

A. B., Augustana College, 1941

AN ABSTRACT OF A THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN CHEMISTRY
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R. UNIV. BIBLIOTHEEK LEIDEN

THEORETICAL

Three possible mechanisms for the methane fermentation of acetic acid are suggested by the findings and reasoning of previous workers in this field. From the work of Omelianskii (5, 6) and Söhngen (7, 8) we would expect a preliminary decomposition of the acetic acid to hydrogen and carbon dioxide, with subsequent reduction of carbon dioxide to methane by the hydrogen. Barker's work (1) indicates that carbon dioxide would be directly reduced to methane and the acetic acid thereby oxidized to carbon dioxide. The reasoning of Buswell and Neave (3) leads to simple decarboxylation as the mechanism.

The first mechanism was based on the similarity of the hydrogen and methane fermentations, but the low concentration of hydrogen found in the gas from the methane fermentation of acetic acid is evidence against this mechanism. If we examine the data of Symons and Buswell (4) we find that only 3.5 liters of methane were formed over a period of 100 days with regular circulation of hydrogen and carbon dioxide through a culture of 2.0 liters total volume. In the same time, such a culture fermenting acetic acid could be expected to form 75 liters of methane. Since the reduction of carbon dioxide is such a slow process, a larger residue of hydrogen would be expected in the gas.

The second mechanism avoids this weakness by implying a direct reduction of the carbon dioxide, without the intermediate stage of free hydrogen. This mechanism seems rather indirect and involved, but parallels that found by Barker in the oxidation of alcohols.

The last mechanism appears to be the simplest and most direct. A similar reaction in vitro is the chemical decarboxylation of sodium acetate with sodium hydroxide. The internal oxidation reduction could be effected by the transfer of the hydrogen atom from the carboxyl to the methyl group.

Evidence against this mechanism is found in Thayer's work (9) specifically and in all work where the gas was analyzed, for no hydrocarbon other than methane has been found. Therefore, if we are to accept this mechanism of simple decarboxylation for acetic acid, it must be as a special case, not applicable to the higher fatty acids.

Barker, Ruben, and Kamen (2) found evidence for the reduction of carbon dioxide in the fermentation of acetic acid through the use of $C^{11}O_2$, but they stated that the radioactive methane might have been due to the presence of methanol carried over with the inoculum. It should also be mentioned that these workers were working with a pure culture of *Methanosarcina methanica*, and that even if methane should be formed by reduction of carbon dioxide in that case, that might not be the predominant mechanism in the general methane fermentation where a mixed culture is used.

This question of the mechanism of fermentation of acetic acid is of more than academic interest. Culture failure is almost invariably accompanied by high concentrations of volatile organic acids, largely acetic. Thus any information concerning this mechanism might lead to methods of treatment or operation which would alleviate or prevent this accumulation of acids and possibly the failure of many cultures.

It may be seen at once that if the carbon of the carbon dioxide were marked isotopically, this question of mechanism could be settled. If either of the mechanisms involving reduction of carbon dioxide were involved, the methane produced should be similarly marked. If the reaction were a simple decarboxylation, the methane should not be so marked.

Radioactive C^{14} was chosen as the most easily handled of the carbon isotopes which could be used for this purpose.

Experimental

The radioactive carbon was obtained thru the Manhattan

District in the form of barium carbonate. This material was converted to sodium carbonate by acidification with perchloric acid and absorption of the carbon dioxide in a solution of sodium hydroxide. The solution resulting was found to yield 3.24×10^5 counts per minute per ml. One ml. of this solution was added to each culture before feeding and incubating.

The fermentations were carried out in 30 ml. round bottom flasks, connected to brine displacement gas collectors. Fifteen ml. of inoculum was taken from a digester which had been fermenting acetic acid for several months. To this was added 1.00 ml. of the radioactive sodium carbonate solution, and 1.0 ml. of 1.0% acetic acid. To the first three cultures, similar portions of acetic acid were added after two days of incubation, and again after the third day. The fourth culture was fed only once and analyzed after two days.

The first culture was analyzed after four days of incubation. The other two were kept in the incubator until they were analyzed.

The culture was acidified and boiled to free the dissolved carbon dioxide and to drive the gas over into the gas collector. The carbon dioxide was absorbed on carbon dioxide free mikobite. The methane was catalytically oxidized to carbon dioxide and this was also absorbed on carbon dioxide free mikobite. The carbonate was washed off of the mikobite with hot water and precipitated with barium nitrate. These operations were carried out in a carbon dioxide free atmosphere.

The precipitated barium carbonate was suspended in ethanol and deposited on an aluminum sample pan, by transferring to a brass tube, at the bottom of which was fastened the sample pan. The alcohol was evaporated under an electric heat lamp.

The sample thus prepared was counted with a conventional type Geiger Mueller counter. The tube used was a pressure

seal type mica window counter (Radiation Counter Laboratories, Mark 1, Model 2A, window thickness—2.19 mg. per sq. cm.). Background counts averaged from 40 to 45 counts per minute.

Correction for geometry was unnecessary, since only one geometry was used, and no correction for absorption by the window was made since the same tube was used throughout. The data of Yankwich, Rollefson, and Norris (10) was used to calculate the correction for self absorption.

The specific activity of the methane was calculated by dividing the total counts by the weight of barium carbonate derived therefrom. The average specific activity of the carbon dioxide was calculated by dividing the average of the initial and final counts by the average of the initial and final equivalent weights of barium carbonate. From these, the activity ratio between the methane and the carbon dioxide, on the basis of the activity of the barium carbonate resulting, was calculated.

The activity ratio was found to vary with time of incubation. It was for this reason that the additional sample with short incubation time was analyzed. The results are listed in Table I below.

TABLE I

Sample No.	Incubation Time (days)	Activity Ratio
1	4	0.0188
2	6	0.0312
3	12	0.0501
4	2	0.0053

DISCUSSION

If the mechanism of the methane fermentation of acetic acid involved the reduction of carbon dioxide to methane, the barium carbonate derived from the methane should have the

same specific activity as the carbon dioxide had during the fermentation. The data show that the activity of the methane is only a few hundredths of that of the carbon dioxide, which makes it immediately apparent that the methane is derived from the acetic acid itself.

The small amount of radioactivity found in the methane might be explained in a number of ways, but the gradual rise in activity with increasing incubation time would seem to indicate that carbon dioxide is assimilated to some extent by the bacteria, forming either cell substance or endogenous compounds, with subsequent decomposition to methane. Whatever explanation we accept for this activity, we must still come to the conclusion that very little methane is formed in this fermentation by direct reduction of carbon dioxide.

This work indicates that the methane is produced by some mechanism other than the reaction with carbon dioxide, and must therefore be derived from the acetic acid. A simple decarboxylation seems to be the most likely mechanism, but is not definitely established. It is still possible that some preliminary condensation might take place, with subsequent decomposition of the condensation product, but this point cannot be proved by this method of attack.

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VITA

The author was born in Moline, Illinois, on July 21, 1919. He attended the Moline schools, and was graduated from the Moline High School in 1936. In September of that year he entered Augustana College in Rock Island, Illinois, from which he received the A. B. degree in 1941. He attended the University of Illinois for the following year, holding a half time appointment with the Illinois State Water Survey. From July, 1942, until February, 1945, he was a special research assistant on a University of Illinois contract with the National Defense Research Council. From February, 1945, to November, 1945, he held the same position on a contract with the Chemical Warfare Service. He then resumed his work with the Illinois State Water Survey. The author has written several unpublished NDRC reports and was co-author of two papers presented at the April, 1947, meeting of the American Chemical Society: Buswell and Sollo, "The Effect of Sodium Sulfate and Sodium Chloride on the Methane Fermentation," and Buswell, Pagano, and Sollo, "An Apparatus for Study of the Continuous Methane Fermentation." Another by Buswell and Sollo, "Methane Fermentation of a Wood Extract," is now in manuscript. The author is a member of Sigma Xi and Phi Lambda Upsilon.